## COLORADO RIVER RECOVERY PROGRAM FY 99 ANNUAL PROJECT REPORT

## RECOVERY PROGRAM PROJECT NUMBER: 97 (CAP-6 SE-NF)

- I. Project Title: Evaluation of interspecific sensitivity to dietary selenium exposure: razorback sucker versus flannelmouth sucker.
- II. Principal Investigator(s): Daniel W. Beyers, Ph.D., Larval Fish Laboratory, Department of Fishery and Wildlife Biology, Colorado State University, Fort Collins, CO 80523; T: 970-491-5475; F: 970-491-5091; E: danb@lamar.colostate.edu
- III. Project Summary: Recently, the impact of changing water quality on razorback sucker has been a concern, but questions remain about why co-occurring native fishes are relatively abundant in the Upper Colorado River Basin. This apparent paradox has led to the hypothesis that the razorback sucker may have different sensitivity to selenium exposure.

To quantify species sensitivity, standard early life-stage toxicity tests were conducted with razorback sucker and the flannelmouth sucker. Larval fish were exposed to gradients of selenium-contaminated water and food organisms. Growth and survival of larvae were monitored over a 28-day period.

A separate study was conducted to compare the sensitivity of razorback sucker and flannelmouth sucker to dissolved selenium. Both species were studied simultaneously using standard 96-hour acute toxicity tests.

- IV. Study Schedule: Initial year: FY99; final year: FY00. The project currently has "ongoing" status with investigations scheduled to be completed by the end of February 2000.
- V. Relationship to RIPRAP:

Colorado River Action Plan-Gunnison II. restore habitat

II.A. restore flooded bottom land habitat

II.A.2.a. preconstruction contaminants screening

II.A.2.d. evaluation

Because selenium contamination is a concern throughout the Colorado River Basin, the proposed research is also relevant to the completed project entitled "Assessment and Prediction of Effects of Selenium Exposure to Larval Razorback Sucker" (CAP-6 SE-1), the ongoing project entitled "Selenium Effects on Larval Razorback Suckers: Field Verification of Laboratory Results" (CAP-6 SE-2) and the following components of the RIPRAP:

General Recovery Program Action Plan II. restore habitat

II.A. restore flooded bottom land habitats

II.A.2. screen high-priority sites for restoration

II.B. support actions to reduce contaminant impacts

II.B.1. evaluate effects of... ...agriculture, and municipal...
...sources of potential contaminants throughout the Upper
Basin

II.C.1. identify what restoration and protection are needed

Green River Action Plan

II. restore habitat

II.A. restore flooded bottom land habitats II.A.2.a. identify and evaluate sites

II.D. support actions to reduce contaminant impacts at Ashley Creek and Stewart Drain

Colorado River Action Plan-Mainstem

II. restore habitat

II.A. restore and manage flooded bottom land habitat

II.A.4. develop and implement levee removal

II.A.4.a. preconstruction contaminants screening

VI. Accomplishment of FY 99 Tasks and Deliverables, Discussion of Initial Findings and Shortcomings:

**Objective 1:** Describe the response of growth and survival of razorback sucker and flannelmouth sucker larvae as functions of selenium concentrations in water and diet.

**Status:** This objective was accomplished. Monocultures of the freshwater algae Chlorella vulgaris (Carolina Biological Supply Company, Burlington, North Carolina) and the rotifer Brachionus calveiflorus (Florida Agua Farms, Dade City, Florida) were successfully cultured. Both organisms were maintained in a series of batch cultures with five target exposure concentrations (control, 25, 50, 100, and 200 µg/L dissolved selenium). Each batch of rotifers was fed algae from the corresponding selenium exposure concentration (e.g., rotifers in the 200 g/L treatment were fed algae from the 200 g/L treatment) one or two times daily. Abundance of rotifers in batch cultures was quantified daily by subsampling. Razorback sucker and flannelmouth sucker larvae were obtained from the Grand Valley Propagation Facility (U.S. Fish and Wildlife Service, Colorado River Fishery Project, Grand Junction, Colorado). Experimental treatments were assigned to replicate aquaria (n=4) using a randomized, balanced 5×2 factorial design with five target exposure concentrations (control, 25, 50, 100, and 200 µg/L) and razorback sucker or flannelmouth sucker as the test organism. This experimental design was equivalent to conducting two toxicity tests simultaneously. In one test, razorback sucker were exposed to selenium in water and diet. In the other, flannelmouth sucker larvae were exposed. Ten larvae were assigned to each exposure aquarium (experimental unit). Larvae were transferred from mass cultures to flow-through aquaria about 24 h before the toxicant metering system was activated. A continuous-flow diluter was used to generate exposure concentrations. The diluter maintained a 0.5 dilution factor and

supplied a volume of 34 ml/min to exposure aquaria. Aquaria were polyethylene vessels having a diameter of 12 cm and height of 15 cm. Depth of test solutions was 9.5 cm. Cool-white fluorescent lamps were the only source of illumination (530 lx), and a 12:12-h light:dark photoperiod was maintained.

Larvae were fed a ration of 1000 contaminated rotifers per day. Survival of fish in each treatment was monitored daily. Growth was quantified at the end of the exposure period by determining the average blotted wet mass and average total length (TL) of fish that survived. Average mass was measured to 0.0001 g; TL to 0.1 mm.

In addition to the food-chain experiment, 96-hour acute toxicity tests were conducted using standard methodology. Target exposure concentrations were control, 10, 20, 40, 80, and 160 mg/L dissolved selenium.

Initial findings: The food chain exposure was conducted as planned. Survival of razorback sucker and flannelmouth sucker in control treatments was 100 and 87.5%, respectively. Preliminary analysis suggests that there was no obvious trend in survival as a function of dissolved selenium concentration in the food chain experiment. Samples of algae, rotifer, and fish have been submitted for chemical analysis for determination of tissue selenium concentrations. Results of these analyses are anticipated to be available in late January. Tissue concentrations are critical for evaluating results of this research. Consequently, in-depth analysis of data have not been conducted.

The acute tests were conducted as planned. Survival of razorback sucker and flannelmouth sucker in control treatments was 100% in both tests. Preliminary analysis suggests that there is an obvious decline in survival as a function of dissolved selenium concentration.

**Shortcomings:** None.

**Objective 2:** Quantitatively compare sensitivity to selenium exposure of razorback sucker to flannelmouth sucker.

**Status and initial findings:** This statistical comparison has not been completed. However, the methodology is relatively straight forward and easily accomplished once all of the data have been received from analytical laboratories.

**Shortcomings:** None.

## VII. Recommendations:

1. Recommend that the program guidance be modified to include an investigation to describe the time course of selenium bioaccumulation in fish food organisms in backwaters. Data that describe the time-dependent relationship between selenium concentrations in backwaters and tissue concentrations in food organisms are needed in order to estimate dietary exposure of fish (adults and larvae) in the field. Because backwaters may fill and drain annually, selenium concentrations in resident food

organisms probably changes over time and this is a major source of uncertainty for evaluating the influence of dietary exposure on wild fish.

- 2. Recommend that the program guidance be modified to include further evaluation of selenium effects on reproductively active razorback sucker. Current research will thoroughly describe effects of selenium exposure on larval razorback sucker, but additional studies are needed to evaluate effects of selenium exposure on reproductively-active adults. Future investigations should emphasize bioaccumulation of selenium in adult fish prior to spawning and monitor survival of resulting embryos and larvae. This research should be conducted under controlled conditions so that selenium exposure is well known and confounding influences are minimized.
- 3. Recommend that the program guidance be modified to include evaluation of influence of fish movement on selenium bioaccumulation. Existing or new radio telemetry investigations of movements of razorback sucker adults could be linked with selenium investigations. Adult fish may move in and out of selenium contaminated areas. Assessments that do not account for this behavior will over estimate effects of selenium on adult fish. Radio telemetry data can be linked to bioaccumulation results using a computer model. The model could simulate accumulation of selenium given fish movements in and out of contaminated areas and estimate reproductive effects.
- VIII. Project Status: Project is considered "ongoing." A draft report is anticipated by the end of February 2000.
- IX. FY 99 Budget Status
  - A. Funds Provided: \$62,296
  - B. Funds Expended: \$47,203
  - C. Difference: \$15,093: \$1,969 indirect cost; \$10,420 to cover costs of selenium analyses that are currently underway; \$2,704 to cover quality assurance and data entry when chemical analyses are complete.
  - D. Percent of the FY 99 work completed, and projected costs to complete: Proposed research is 95% complete; remaining budget is adequate to finish this component.
  - E. Recovery Program funds spent for publication charges: \$0.00
- X. Status of Data Submission (Where applicable): NA
- XI. Signed: <u>Daniel W. Beyers, Ph.D.</u> <u>3 December 1999</u> Principal Investigator Date